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Effect of NPK and foliar nutrition on growth, yield and chemical constituents in *Nigella sativa* L.

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Abstract
The seeds of *Nigella sativa* L**.** (Family *Ranunculaceae*) have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. Plant nutrition one of the most important factors that increase plant production. Thus, the main objective of the present investigation was to study the effect of different levels of NPK fertilizers, foliar nutrition and their interactions on the morphological and biochemical contents of *Nigella sativa* L**.** The effect of NPK and foliar nutrition on the growth [Plant height (cm), leaf number (plant^{-1}), branch number (plant^{-1}), capsule number (plant^{-1}), herb dry weight (plant^{-1}) and seed yield (plant^{-1})] was measured and quantitative analysis of fixed oil, total carbohydrate, soluble sugars and nutrient content were performed. The most effective rate was $N_3P_3K_3$ x foliar nutrition interaction, resulting in a positive increase in vegetative growth. The highest values of vegetative growth characters were 27.7, 41.4 cm (plant height); 55.4, 51.9 (leaf number); 10.2, 11.7 plant^{-1} (branch number); 15.5, 20.8 plant^{-1} (capsule number); 47.1, 49.4 g plant^{-1} (herb dry weight); 4.3, 4, 7 g plant^{-1} (seed yield) during the first and second seasons respectively. As well as $N_3P_3K_3$ x foliar nutrition led to higher biochemical contents than the control. The highest values of chemical contents were 22.9 and 25.1% (fixed oil); 33.0, 30.1 % (total carbohydrate); 16.9, 8% (soluble sugars); 23.7 and 24.8 % (protein); 3.8 and 4 % (N); 0.4 and 0.4 % (P); 1.2 and 1.8 % (K) during the first and second seasons respectively.

Key words: Nigella. Sativa L.*,* NPK, foliar nutrition, morphology and biochemical contents.

1. Introduction
Nigella sativa L. belongs to family *Ranunculaceae* is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *Nigella sativa* L. have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Prophetic Medicine. It has been widely used as antihypertensive, liver tonics, diuretics, digestive, antidiarrheal, appetite stimulant, analgesics, antibacterial and in skin disorders [1]. Follow in all ref quoted. Plant nutrition one of the most important factors that increase plant production. Nitrogen (N) is the most recognized in plant for its presence in the structure of the protein molecule. Accordingly, N plays an important role in synthesis of the plant constituents through the action of different enzymes [2]. Seeds have the highest concentration of P in a mature plant, and P is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid. P aids in root development, flower initiation, seed and fruit development. P_2O_5 has been shown to reduce disease incidence in some plants and has been found to improve the quality of certain crops [3]. Potassium (K) is an important macro-nutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation [4, 5]. Micro-nutrients are involved in all metabolic and cellular functions. Plants differ in their need for micro-nutrients. Several of these elements are redox-active that make them essential as catalytically active co-factors in enzymes, others have enzyme-activating functions, and yet others fulfill a structural role in stabilizing proteins [6]. Application of trace elements improved the performance of the plants; increased leaf size and yield of foxglove plant [7]. Hornok [8]

indicated that NP fertilization not only effective on the quantity of vegetative and generation mass, but on the oil content of dill (*Anethum graveolens* L.). The application of 100 kg N and 26 kg P per hectare produced the highest biomass and oil yields and NPK uptake of davana (*Artemisia pallens* Wall.) [9].The highest yields of inflorescence and essential oil of chamomile (*Chamomilla recutita* (L.) were achieved when the ratio between the major nutrients N: P was 1:1 [10]. High amount of NP (2.0 and 4.0 g pot^{-1}) increased plant height, dry mass, and flower head yield of gum (*Grindelia camporum* Greene plants [11]. Foliar application improved the performance of the plants; increased leaf size and yield of foxglove plant [7]. Kandeel [12] reported that using foliar application at 2000 mg L^{-1} + NPK had a significant effect on plant height, fresh weight, dry weight, fruit yield and oil content of parsley (*Petroselinum crispum* Mill). NPK + foliar nutrition had a significant effect on anise, coriander and sweet fennel plants which positively affect growth and chemical constituent's of these three plants grown under arid regions in Egypt [13]. The main objective of the present investigation was to study the effect of different levels of NPK fertilizers, foliar nutrition and their interactions on the morphological and biochemical contents of *Nigella. Sativa* L**.**

2. **Materials and methods**

2.1. Experimental

The present study was carried out in the Experimental Farm, Faculty of Agriculture, Ain Shams University, located at Shubra El-Kheima, Kalubia, Egypt, during two successive seasons of 2006 / 2007 and 2007 / 2008. *Nigella sativa* L. seeds were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. In the first week of November during both seasons seeds were sown in plastic pots (30 cm diameter and 50 cm height), 10 seeds per pot. The viability of seeds was approximately 92%. In the third week of December during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried clay soil. Physical and chemical properties of the soil used in this study were determined according to Jackson [14,15] and presented in Table 1.

Table 1: Physical and Chemical properties of the soil (average of 3 samples from 30 -50 cm depth)

Clay (%)		Silt (%)		Sand (%)			Texture	
67.0		9.0		24.0			Clay	
Soluble cations (mg/100 soil)				Soluble anions (mg/100 soil)				
Ca	Mg	Na	K	Co_3	HCO_3	Cl	SO_4^-	
106.0	62.0	41.0	39.8	-	2.0	5.0	-	106.0
OM (%)	SP (%)	$CaCo_3$ (%)	pH	EC (dS m^{-1})	NO_3 (ppm)	P (ppm)	CO (mg L^{-1})	SAR
1.4	31.8	4.8	7.2	1.8	20.1	1.5	-	4.5
OM= Organic Matter, SP= Saturation Pecentage, EC= Electronic Conductivity, SAR= Sodium Adsopation Ratio.								

Eight weeks after sowing the seedlings were thinned to three plants per pot. Pots were divided into 2 main groups. The first group was subjected to different levels of NPK (0:0:0, 1:1:1, 2:2:2 and 3:3:3). The second group was subjected to the same treatments of NPK but foliar nutrition was added as foliar spray. All agricultural practices were practiced according to the main recommendations by the Egyptian Min. Agric. The sources of NPK fertilizers were ammonium sulphate (N, 20.5%), calcium super phosphate (P_2O_5, 15.5 %) and potassium sulphate (48% K_2O). Foliar nutrition was solution commercially known as Agronal, which consists of the following minerals: N (120 mg L^{-1}) – P_2O_5 (40 mg L^{-1}) – K_2O (40 mg L^{-1}) – Mg (2 mg L^{1}) – S (2 mg L^{-1}) - Fe (1200 mg L^{-1}) – Zn (1200 mg L^{-1}) – Mn (1000 mg L^{-1}) – Cu (500 mg L^{1}) – Ni (1 mg L^{-1}) – CO (1mg L^{-1}).

2.2. Harvesting

At fruiting stage, the plants were harvested at the end of the two seasons. Vegetative growth characters measurements [Plant height (cm), leaf number ($plant^{-1}$), branch number ($plant^{-1}$), capsule number ($plant^{-1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$)] were recorded.

2.3. Total carbohydrate (TC) and total soluble sugars (TSS) determination

TSS concentrations in seeds (collected at the end of the first and second seasons of each treatment) were determined according to Ciha [16] with some modifications. Samples of 100 mg were homogenized with 10 ml of extracting solution [glacial acetic acid: methanol (or sulphoric acid as 1n): water, 1:4:5, v/v/v]. The homogenate was centrifuged for 10 min at 3.000 rpm and the supernatant was decanted. The residue was re - suspended in 10 ml of extracting solution and centrifuged another 5 min at 3.000 rpm. The supernatant was decanted, combined with the original extract and made up to 50 ml with water. For measurement of total carbohydrate and TSS, a phenol-sulfuric acid assay was used [17]. A volume of

0.5 ml of 5% (v/v) phenol solution and 2.5 ml of concentrated sulfuric acid were added to 0.5 ml aliquots. The mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometer at 490 nm.

2.4. *Fixed oil (FO), nutrients and protein determination*

FO extraction: 50 g of seeds were crushed to coarse powder and extracted with petroleum ether (40-60 ^{o} C) in a Soxhlet apparatus [18].

N, protein, P and K (in the leaves) of both seasons of each treatment were determined using the methods described by the AOAC [18] as follows: The washed and dried materials were ground to fine powder with mortar and pestle and used for dried ash. For analysis of K the powdered plant material (0.2 g) was taken in precleane and constantly weighed silica crucible and heated in muffle furnace at 400 ^{0}C till there was no evolution of smoke. The crucible was cooled in desiccator's at room temperature. The ash totally free from carbon moistened with Conc. H_2SO_4 and heated on Hot plate till fumes of sulphuric acid get evolved the silica crucible with sulphated ash was again heated at 600 ^{0}C in muffle furnace till weight of sample was constant (3-4 hrs) one gram sulphated ash were taken in beaker which dissolved in 100 ml 5 % conc. HCl to obtain solution for determination of K through flame photometry, standard solution of each mineral was prepared and calibration curve drawn for K element using flame photometry. For determination of protein and Nitrogen using Micro Kjeldahl method, 1 g of plant sample taken in a Pyrex digestion tube and 30 ml of conc. H_2SO_4 carefully added, then 10 g potassium sulphate and 14 gm copper sulphate, mixture is placed on sand both on a low flame just to boil the solution, it was further heated till the solution becomes colorless and clear, allowed to cool, diluted with distilled water and transferred into 800 ml Kjeldahl flask, washing the digestion flask, Three or four pieces of granulated zinc and 100 ml of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N sulphuric acid was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using Methyl Red indicator for determination of nitrogen, which in turn give the protein content. For determination of phosphorous 2 g sample of plant material taken in 100 ml conical flask two spoons of Darco-G-60 is added followed by 50 ml of 0.5 M $NaHCO_3$ solution, next flask was corked, and allowed for shaking for 30 min. on shaker. the content was filtered and filtrate was collected in flask from which 5 ml filtrate was taken in 25 ml volumetric flask to this 2 drops of 2, 4- paranitrophenol and 5 N H_2SO_4 drop by drop was added with intermittent shaking till yellow color disappear, content was diluted about 20 ml with distilled water and then 4 ml ascorbic acid was added then the mixture was shacked well and the intensity of blue color at 660 nm on colorimeter was measured. The absorbencies were compared and concentrations of phosphorous using standard value were calculated.

2.5. *Statistical analysis*

In these experiments, two factors were considered: NPK and foliar nutrition. For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual plants. The experimental design followed a complete random block design. According to Snedecor [19], the averages of data were statistically analyzed using 2-way analysis of variance (ANOVA -2) and the values of least significant difference (LSD) at 5%.

3. Results and discussion

3.1 Effect of NPK, foliar nutrition and their interactions on the growth characters

NPK and foliar nutrition affected in plant morphology (Table 2). Plant growth characters such as [Plant height (cm), leaf number (plant^{-1}), branch number (plant^{-1}), capsule number (plant^{-1}), herb dry weight (g plant^{-1}) and seed yield (g plant^{-1})] were significantly affected by changes in NPK fertilization + foliar nutrition treatments. Thus the various growth characters in general increased under the various NPK fertilization levels + foliar nutrition compared with NPK fertilization treatments. Highest values of plant growth characters were obtained in the $N_3P_3K_3$+ foliar nutrition treatment with the values of 27.7, 41.4 cm (plant height); 55.4, 51.9 (leaf number); 10.2, 11.7 plant^{-1} (branch number); 15.5, 20.8 plant^{-1} (capsule number); 47.1, 49.4 g plant^{-1} (herb dry weight); 4.3, 4,7 g plant^{-1} (seed yield) during the first and second seasons, respectively compared with control and other treatments (Table 2).

3.2. Effect of NPK, foliar nutrition and their interactions on the fixed oil content

Fixed oil content increased with NPK, foliar nutrition and their interactions (Table 3). However, the highest fixed oil content resulted from $N_3P_3K_3$ + foliar nutrition treatment with values of 22.9 and 25.1% for the first and second season compared with control (15.1 and 17.3 %).

Table 2: Effect of NPK, foliar nutrition and their interactions on the growth characters

Treatments		Growth Characters											
		Plant height (cm)		Branch no. (plant $^{-1}$)		Leaf no. (plant $^{-1}$)		Capsule no. (plant $^{-1}$)		Dry weight (g plant $^{-1}$)		Seed yield (g plant $^{-1}$)	
		Seasons		Seasons		Seasons		Seasons		Seasons		Seasons	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without Foliar Nutrition	$N_0P_0K_0$	18.2	20.5	6.7	5.8	17.9	25.5	3.1	5.1	20.9	22.8	3.6	4.0
	$N_1P_1K_1$	19.9	25.1	7.5	5.8	27.6	32.5	4.5	7.4	37.7	38.7	3.6	4.1
	$N_2P_2K_2$	22.7	28.7	8.3	7.5	36.8	42.1	13.1	11.7	40.1	41.8	3.7	4.2
	$N_3P_3K_3$	23.9	34.7	9.2	9.0	48.0	50.3	13.7	18.7	42.7	42.1	3.8	4.3
Overall Without		21.2	27.3	7.9	7.0	32.6	37.6	8.6	10.7	35.4	36.3	3.7	4.2
With Foliar Nutrition	$N_0P_0K_0$	20.4	23.1	8.0	8.6	29.9	32.5	4.5	6.0	24.7	26.5	3.7	4.2
	$N_1P_1K_1$	23.2	28.3	8.5	9.8	32.5	35.1	4.9	9.3	40.9	41.8	3.9	4.3
	$N_2P_2K_2$	24.5	34.5	9.2	10.9	41.9	46.5	14.2	19.5	45.9	47.8	4.2	4.5
	$N_3P_3K_3$	27.7	41.4	10.2	11.7	55.4	51.9	15.5	20.8	47.1	49.4	4.3	4.7
Overall With		24.0	31.8	9.0	10.3	39.9	41.0	9.8	13.9	39.7	41.4	4.0	4.4
Overall NPK	$N_0P_0K_0$	19.3	21.8	7.5	7.2	23.9	29.0	3.8	5.6	22.8	24.7	3.7	4.1
	$N_1P_1K_1$	21.6	26.7	8.0	7.8	30.1	33.8	4.7	8.4	39.3	40.3	3.8	4.2
	$N_2P_2K_2$	23.6	31.6	8.9	9.2	39.4	44.3	13.7	15.6	43.0	44.8	4.0	4.4
	$N_3P_3K_3$	25.8	38.1	9.7	10.4	51.7	51.1	14.6	19.8	44.9	45.8	4.1	4.5
LSD													
NPK		2.1	2.5	1.2	1.3	4.1	4.2	1.0	1.0	3.5	3.7	0.2	0.2
Foliar Nutrition		1.2	2.2	1.0	1.1	3.2	3.4	0.9	1.1	2.3	2.5	0.1	0.1
NPK X Foliar Nutrition		2.3	2.4	1.1	1.3	4.5	4.8	1.1	1.3	4.4	5.6	0.3	0.3

3.3. Effect of NPK, foliar nutrition and their interactions on the total carbohydrate and soluble sugars content

Total carbohydrate and soluble sugars content increased with NPK fertilization, foliar nutrition and the NPK fertilization × foliar nutrition interaction (Table 3). However, the highest total soluble sugars content resulted from $N_3P_3K_3$ + foliar nutrition treatment with the values of 33.0, 30.1 % (total carbohydrate) and 16.9, 8% (soluble sugars) during the first and second seasons prospectively compared with control and other treatments.

3.4. Effect of NPK, foliar nutrition and their interactions on the crude protein content

The accumulation of protein in black seed plants leaves was promoted by applying various levels of NPK, foliar nutrition and their interactions (Table 3). The highest protein content resulted from $N_3P_3K_3$ + foliar nutrition treatment with the values of 23.7 and 24.8 % during the first and second seasons.

3.5. Effect of NPK, foliar nutrition and their interactions on mineral content

Addition of NPK ameliorated the increase in NPK contents (%) with increasing NPK fertilization. Control ($N_0P_0K_0$) treatment resulted in the lowest nutrient accumulation while the highest mineral content was observed in the $N_3P_3K_3$ + foliar nutrition treatment with the values of 3.8 and 4.0 % (N); 0.4 and 0.4 % (P); 1.2 and 1.8 % (K) during the first and second seasons, respectively.

The positive effects of these treatments (NPK, foliar nutrition and their interactions) may be due to the important physiological role of N; N plays an important role in synthesis of the plant constituents through the action of different enzymes activity and protein synthesis [2] that was reflected on an increase in growth parameters and chemical constituents of black seed plants. The obtained results are in accordance with those obtained by previous literature. N is a necessary component of several vitamins. N improves the quality and quantity of dry matter in leafy plants and protein in grain crops [3]. Increase the N fertilizer caused a significant increase in the seed yield of Japanese mint (*Mentha arvensis* L) [20, 21]. N fertilization increased the vegetative growth, fixed oil, total carbohydrate, soluble sugars and NPK content of *Nigella sativa* L. plants [22].

Zheljazkov [23] established that vegetative growth and oil of *Mentha piperita* and *Mentha arvensis* were increased as N fertilizer increase [24]. Arabaci [25] found that N fertilizer increased the amount of green herb yield, drug herb yield, drug leaves, oil of basil (*Ocimum basilicum* L.). N fertilization increased the dry weight of *Mentha.* x *piperita*, linalool chemotype [26]. P leads to enhanced herb and essential oil yields of different mint species [27]. The P had a stimulating effect on the growth parameters, total carbohydrate, soluble sugars,

mineral contents and on the percentage of oil production from chamomile flowers compared with the control [28]. Protein results may be due to N which has an influence on the ribosome structure and the biosynthesis of some hormones (gibberellines, auxins and cytokinins) involved in protein synthesis [2]; P activates co-enzymes for amino acid production used in protein synthesis [29].

Table 3: Effect of NPK, foliar nutrition and their interactions on the chemical constituents

Treatments		Chemicals Constituents (%)													
		Fixed oil		T. Carbohydrate		S. Sugars		N		Protein		P		K	
		Seasons		Seasons		Seasons		Seasons		Seasons		Seasons		Seasons	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without Foliar Nutrition	$N_0P_0K_0$	15.1	17.3	17.0	19.7	7.3	2.8	2.9	3.1	17.8	19.4	0.1	0.1	0.6	0.9
	$N_1P_1K_1$	15.3	19.2	24.1	24.0	9.3	4.3	3.2	3.3	20.0	20.3	0.2	0.2	0.7	1.1
	$N_2P_2K_2$	17.6	21.0	24.2	24.0	9.5	5.1	3.4	3.5	21.3	21.9	0.3	0.3	0.8	1.3
	$N_3P_3K_3$	19.8	22.0	26.3	25.2	11.0	6.1	3.6	3.8	22.2	23.8	0.3	0.3	0.9	1.4
Overall Without		17.0	18.9	22.9	23.3	9.3	4.6	3.3	3.4	20.3	21.4	0.2	0.2	0.8	1.2
With Foliar Nutrition	$N_0P_0K_0$	17.7	19.0	21.0	22.5	9.3	3.1	3.2	3.2	19.7	20.6	0.2	0.2	0.9	1.2
	$N_1P_1K_1$	19.2	21.0	25.4	26.4	12.7	4.1	3.4	3.5	20.9	21.6	0.3	0.3	1.1	1.4
	$N_2P_2K_2$	21.6	23.0	27.1	27.2	14.2	6.1	3.5	3.6	21.6	21.6	0.3	0.3	1.1	1.6
	$N_3P_3K_3$	22.9	25.1	33.0	30.1	16.9	8.0	3.8	4.0	23.7	24.8	0.4	0.4	1.2	1.8
Overall With		20.4	22.0	26.6	26.6	13.3	5.3	3.5	3.6	21.5	22.2	0.3	0.3	1.1	1.5
Overall NPK	$N_0P_0K_0$	16.4	18.2	19.0	21.1	8.3	2.95	3.1	3.2	18.8	20.0	0.2	0.2	0.8	1.1
	$N_1P_1K_1$	17.3	20.1	24.8	25.2	11.0	4.2	3.3	3.4	20.5	21.0	0.3	0.3	0.9	1.3
	$N_2P_2K_2$	19.6	22.0	25.7	25.6	11.9	5.6	3.5	3.6	21.5	21.8	0.3	0.3	1.0	1.5
	$N_3P_3K_3$	21.4	23.6	29.7	27.7	14.0	7.1	3.7	3.9	23.0	24.3	0.4	0.4	1.1	1.6
LSD															
NPK		1.4	1.3	1.2	1.2	1.2	1.2	0.2	0.2	0.9	0.9	0.1	0.1	0.1	0.1
Foliar Nutrition		1.3	1.2	1.1	0.9	1.1	1.1	0.1	0.1	0.8	0.8	0.1	0.1	0.1	0.1
NPK X Foliar Nutrition		1.5	1.5	1.5	1.3	1.3	1.3	0.3	0.3	1.2	1.2	0.1	0.1	0.1	0.1

Potassium (K) is an important macro-nutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation such as enzyme of essential oil synthesis [5]. Trace elements are redox-active that makes them essential as catalytically active co-factors in enzymes, others have enzyme-activating functions, and yet others fulfill a structural role in stabilizing proteins [6]**.** Hussien [30] reported that NPK fertilization was more effective on the dill oil. It was established that plant height, branching and essential oil content were increased with increasing NP fertilizer rates. However, plant leaves were not significantly affected by the increase of NPK rates [23]. Fresh material and oil yields of peppermint (*Mentha X piperita* L.) were increased by the increase in NPK levels [31]. NPK treatments produced the highest growth and oil of garden thyme (*Thymus vulgaris* L.) compared with the control treatment [32]. Spraying of foliar nutrition under sandy soil conditions resulted in a significant increase in vegetative characters, oil, NPK and total carbohydrate content of *Trachyspermum ammi* L [33]. Nasiri [28] showed that flower yield, oil (% and yield) increased by foliar nutrition application compared with control untreated. Oil, growth and yield of onion plants significantly increased by the application of foliar nutrition compared with control plants [34]. NPK fertilization + foliar nutrition increased the vegetative growth, fixed oil, total carbohydrate, soluble sugars, protein N, P and K of some medicinal *Apiaceae* plants [35]. Increasing the essential minerals according to the NPK, foliar nutrition and their interactions treatments may be due to the increase in the dry matter of plant materials [2, 3, 12, 35]. The effect of NP, foliar nutrition and their interaction*s* treatments on oil may be due to its effect on enzyme activity and metabolism of oil production in peppermint plant [36]. The results of fixed oil agree with those obtained by Khalid [22] on *Nigella sativa* L. plant; Espinosa [29] who indicated that NPK and foliar nutrition plays an important role in various metabolism processes such as fatty acid (fixed oil) synthesis. These findings agree that obtained by the essential oil of *Nigella sativa* as antibacterial against the used bacterial strains such as Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter cloacae and Salmonella enteric [37] and as inhibitor of copper in cooling water [38].

Conclusion

It may be concluded that:

1. NPK + foliar nutrition had a significant effect on growth characters of *Nigella sativa* L**.** plants.
2. NPK + foliar nutrition had a significant effect on chemical constituent's of *Nigella sativa* L**.** plants.

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